J. RADIAT. RES., 42, 255–264 (2001)

Dependence of Malformation upon Gestational Age and Exposed Dose of Gamma Radiation

SUNG HO KIM[1], JONG HWAN LEE[1], HEON OH[1], SE RA KIM[1], CHA-SOO LEE[2], SUNG KEE JO[3], TAE-HWAN KIM[4] and YUN-SIL LEE[4*]

[1]College of Veterinary Medicine, Chonnam National University, Kwangju, Korea;
[2]College of Veterinary Medicine, Kyungpook National University, Taegu, Korea;
[3]Food Irradiation Team, KAERI, Taejeon, Korea;
[4]Laboratory of Radiation Effect, Korea Cancer Center Hospital, Seoul 139–706, Korea

(*Received, October 4, 2000*)
(*1st. Revision received, January 9, 2001*)
(*2nd. Revision received, April 2, 2001*)
(*3rd. Revision received, April 30, 2001*)
(*Accepted, May 15, 2001*)

Dose relationship/Gestational age/Radiation/Mice/Abnormalities

In order to evaluate the importance of gestational age and the dose-incidence relationship by gamma radiation, pregnant ICR mice at gestational days from 2.5 to 15.5 days post-coitus (p.c.) were exposed to a single dose of 2.0 Gy and also at day 11.5 after conception, which was the most sensitive stage for the induction of major congenital malformations. The animals were sacrificed on day 18 of gestation and the fetuses were examined for mortality, growth retardation, changes in head size and other morphological abnormalities. The only demonstrable effect of irradiation during the pre-implantation period was an increase in prenatal mortality. Resorptions were maximal on exposure at day 2.5 after conception. The pre-implantation irradiated embryos which survived did not show any major fetal abnormalities. A small head, growth retardation, a cleft palate, dilatation of the cerebral ventricle, a renal pelvis, and abnormalities of the extremities and tail after exposure were prominent during the organogenesis period, especially on day 11.5 of gestation. As for the dose-incidence relationship, the incidence of a small head, growth-retarded fetuses, a cleft palate, dilatation of cerebral ventricle and abnormalities of the extremities in live fetuses rose as the radiation dose increased. The result indicated that the late period of organogenesis in the development of the brain, skull and extremities of a mouse was a particularly sensitive phase. The threshold doses of radiation that induced a cleft palate and dilatation of the cerebral ventricle, and abnormal extremities were between 1.0 and 2.0 Gy, and between 0.5 and 1.0 Gy, respectively.

*Corresponding author: Phone; 82–2–970–1325, Fax; 82–2–977–0381, E-mail; yslee@mail.kcch.re.kr

INTRODUCTION

The irradiation of mammalian embryos can produce a spectrum of morphological changes, ranging from a temporary stunting of growth to severe organ defects and death[1]. During the period of major organogenesis, mammalian embryos are extreamly susceptible to radiation-induced gross anatomic abnormalities; this period spans 7 to 12 days p.c. in mice, corresponding to about 14 to 50 days in humans[2]. The induced abnormalities depend on the organs undergoing proliferation and differentiation at the time of irradiation, the stage of proliferation and differentiation and radiation dose[3–5].

The effect of irradiation during the early period of murine development, such as the one-cell to blastocyst stage, has been extensively studied *in vitro* by Streffer and co-workers[6,7] and *in vivo* by Russell, Rugh and others[8–11]. In the past, the induction of malformations resulting from exposure to radiation during major organogenesis and early fetal periods had received considerable attention in radiation embryology[8,12–16], and still continues to a focus of study[17–20]. However, Mole argued in a review that the concept of critical periods based on a marked response at high doses of radiation may not be applicable to lower doses[21]. Furthermore, although numerous studies on radiation teratology have been published[22], there is relatively little information available on the relationship between radiation dose and the incidence of specific abnormalities. This led us to carry out a systematic study on the highly sensitive prenatal periods and dose-incidence related to radiation.

MATERIAL AND METHODS

Animals

ICR mice maintained under controlled temperature, light conditions, on standard mouse food and water *ad libitum* were used in the experiment. Virgin females and males, 10–12 weeks of age, were randomly mated overnight. Females with a vaginal plug were separated in the morning and marked as 0 day pregnant. All of the mice were killed on day 18 p.c. by cervical dislocation.

Irradiation

The pregnant mice were exposed to 2.0 Gy gamma-radiation at a dose-rate of 10 Gy/min on any one gestation day from 2.5 to 15.5 days p.c., and the dose-incidence relationships with 0.5, 1.0, 2.0 and 4.0 Gy of gamma-radiation were investigated on day 11.5 after conception.

Prenatal mortality

Uterine horns were opened and the total number of implantations including resorption, embryonic death and fetal death were examined. These were: (A) resorptions including (a) implantation failure in which the implantation site was shown by a rudimentary fleshy mass, but not a full placentum, and (b) a placentum without attached embryonic rudiments. (B)

Embryonic death: a partly formed embryo attached to the placental disc. (C) Fetal death: fully formed dead fetuses, distinguished by darker color, and macerated fetuses that were pale in color and soft to touch. Pre-implantation loss with no identifying mark on the uterine wall, if any, was not counted in this study.

Fetal anomalies

Live fetuses were removed from the uterus and cleaned, and any externally detectable developmental anomalies were examined. Fetuses were weighed individually, and the mean fetal weight of the individual litter of the group was calculated. Fetuses weighing less than two standard deviations of the mean body weight of the control group were considered to be growth-retarded. The body length was measured from the tip of the snout to the base of the tail. The longitudinal distance from the tip of the snout to the base of the skull was recorded as the head length. The distance between the two ears was recorded as the head width. All measurements were made with vernier callipers, and all fetuses were checked for external malformations under a dissection microscope. The fetuses were fixed in Bouin's solution, then stored in 70% ethanol. The presence of visceral malformations was determined by Wilson's cross-sectional technique[23], and Alizarin red-S and alcian blue staining was used to examine

Table 1. Observation on mouse fetuses 18 days after exposure to 2 Gy gamma-radiation on different gestation days.

Observations	Exposure day p.c.					
	Control	2.5	5.5	7.5	11.5	15.5
No. of mother	6	6	6	6	6	6
No. of implants	74	74	76	82	86	74
No. of embryonic death	3	1	6	15[a]	5	3
No. of fetal death	2	0	1	2	1	0
No. of resorption	0	48[d]	10[b]	20[d]	0	0
Prenatal mortality						
No. (%)	5(6.76)	49(66.22)[d]	17(22.37)[a]	37(45.12)[d]	6(6.98)	3(4.05)
Live fetuses	69	25	59	45	80	71
GRF						
No. (%)	5(7.25)	2(8)	41(69.49)[d]	30(66.67)[d]	80(100)[d]	22(30.99)[c]
Body weight (g)	1.59 ± 0.09	1.61 ± 0.12	1.33 ± 0.06[d]	1.26 ± 0.23[d]	0.92 ± 0.08[d]	1.44 ± 0.01[d]
Body length (cm)	3.45 ± 0.63	3.60 ± 0.93	3.23 ± 0.42[a]	3.10 ± 0.40[b]	2.71 ± 0.22[d]	3.21 ± 0.65[a]
Head length (cm)	1.15 ± 0.05	1.16 ± 0.02	1.12 ± 0.01[d]	1.07 ± 0.04[d]	1.02 ± 0.04[d]	1.17 ± 0.02[a]
Head width (cm)	0.84 ± 0.02	0.84 ± 0.02	0.79 ± 0.01[d]	0.79 ± 0.07[d]	0.72 ± 0.02[d]	0.81 ± 0.01[d]
Incidence of decreased head length	2.90	4.0	3.39	44.44	72.5	1.41
Incidence of decreased head width	2.03	8	49.15	53.33	98.75	28.17

GRF: Growth-retarded fetuses, calculated as the number of growth-retarded fetuses/ total number of live fetuses. Fetuses weighing less than two standard deviations of mean body weight of the control group were considered as growth retarded.

A head width or length of less than two standard deviations of mean control value was defined as decreased head width or length.

[a-d] Difference from the control. [a]$p < 0.05$, [b]$p < 0.005$, [c]$p < 0.001$, [d]$p < 0.0001$.

any skeletal malformations[24].

Statistics

A statistical analysis was carried out by the Mann-Whitney U-test, in which $p < 0.05$ was considered to be significant.

RESULTS

A significant increase in prenatal mortality was observed when irradiated at days 2.5 p.c. and 5.5 p.c. of post implantation, the maximum effect being on day 2.5 p.c. Early organogenesis (day 7.5 p.c.) was also highly sensitive to the effect; the prenatal mortality was increased to 6.7 fold when compared to control mice. Exposure at the late organogenesis and fetal stages did not result in any significant increase in mortality (Table 1), suggesting that 2 Gy did not affect the radiation-induced mortality. Of course, in a dose-incidence response study (Table 2), a significant increase in mortality was seen only upon exposure to 4 Gy at 11.5

Table 2. Observation on mouse fetuses 18 days after exposure to different doses of gamma-rays on 11.5 day of gestation.

Observations	Dose (Gy)				
	0	0.5	1.0	2.0	4.0
No. of mother	6	7	7	6	6
No. of implants	74	109	116	86	93
No. of embryonic death	3	3	0	5	3
No. of fetal death	2	1	0	1	6
No. of resorption	0	5	6	0	24[b]
Prenatal mortality					
No. (%)	5(6.76)	9(8.26)	6(5.17)	6(6.98)	33(35.48)[b]
Live fetuses	69	100	110	80	60
GRF					
No. (%)	5(7.25)	41(41)[b]	67(60.91)[b]	80(100)[b]	60(100)[b]
Body weight (g)	1.59 ± 0.09	1.41 ± 0.12[b]	1.34 ± 0.15[b]	0.92 ± 0.08[b]	0.62 ± 0.11[b]
Body length (cm)	3.45 ± 0.63	3.53 ± 0.13	3.41 ± 0.16	2.71 ± 0.22[b]	2.50 ± 0.24[b]
Head length (cm)	1.15 ± 0.05	1.13 ± 0.05[a]	1.09 ± 0.05[b]	1.02 ± 0.04[b]	0.89 ± 0.06[b]
Head width (cm)	0.84 ± 0.02	0.80 ± 0.05[b]	0.76 ± 0.04[b]	0.72 ± 0.02[b]	0.61 ± 0.03[b]
Incidence of decreased head length	2.90	48	79.09	72.5	100
Incidence of decreased head width	2.03	3	26.36	98.75	100

GRF: Growth-retarded fetuses, calculated as the number of growth-retarded fetuses/ total number of live fetuses. Fetuses weighing less than two standard deviations of mean body weight of the control group were considered as growth retarded.

A head width or length of less than two standard deviations of mean control value was defined as decreased head width or length.

[a,b] Difference from the control. [a]$p < 0.005$, [b]$p < 0.0001$.

Table 3. Malformations of fetuses 18 days after exposure to 2 Gy gamma-radiation on different gestation days.

	Exposure day p.c.					
	Control	2.5	5.5	7.5	11.5	15.5
External malformation						
Fetus examined	69	25	59	45	80	71
Ablepharon	0	0	0	1(2.22)	0	0
Micrognathia	0	0	0	1(2.22)	0	0
Gastroschisis	0	0	0	1(2.22)	0	0
Omphalocele	0	0	0	2(4.44)	0	0
Kinky tail	0	0	1(1.69)	2(4.44)	14(17.5)	0
Branchyury	0	0	0	1(2.22)	3(3.75)	1(1.41)
Rudimentry tail	0	0	0	1(2.22)	0	0
Digits	0	0	0	12(26.67)	72(90)	1(1.41)
Anal atresia	0	0	0	0	1(1.25)	0
Internal malformation						
Fetuses examined	35	13	31	22	41	37
Dilatation of cerebral ventricle	0	0	2(6.45)	9(40.91)	26(63.41)	0
Stenosis of nasal cavity	0	0	0	1(4.55)	1(2.44)	0
Cleft palate	0	0	0	0	15(36.59)	0
Dextrocardia	0	0	0	3(13.64)	0	0
Levorotation of heart	0	0	1(3.23)	4(18.18)	2(4.83)	0
Abnormal lobation of lung	0	0	0	3(13.64)	1(2.44)	0
Detect of diaphragm	0	0	0	1(4.55)	0	0
Diaphragmatic hernia	0	0	0	3(13.64)	0	0
Dilatation of renal pelvis	0	2(15.38)	10(32.26)	7(31.82)	3(7.32)	0
Skeletal malformation						
Fetuses examined	34	12	28	23	39	34
Deformity of occipital bone	0	0	1(3.57)	2(8.70)	1(2.56)	0
Splitting of cervical vertebrae	0	0	0	4(17.39)	0	0
Abnormal arrangement of cervical vertebrae	0	0	0	1(4.35)	0	0
Abnormal ossification of coccygeal vertebrae	0	0	0	0	1(2.56)	0
Fusion of lumbar vertebrae	0	0	0	0	1(2.56)	0
Abnormal arrangement of lumbar vertebrae	0	0	0	1(4.35)	0	0
Fusion of thoracic vertebrae	0	0	0	0	2(5.13)	0
Absence of ribs	0	0	0	2(8.70)	0	0
Fusion of ribs	0	0	0	4(17.39)	0	0
Bifurcation of ribs	0	0	0	2(8.70)	0	0
Shortening of ribs	0	0	0	2(8.70)	0	0
Displasia of sternebrae	0	0	0	0	1(2.56)	0
Missing of sternebrae	0	0	0	0	1(2.56)	0
Hypoplasia of sternebrae	0	0	0	3(13.04)	3(7.69)	0
Curvature of tibia	0	0	0	0	1(2.56)	0
Absence of metatarsal bone	0	0	0	0	5(12.82)	0
Absence of metacarpal bone	0	0	0	0	15(38.46)	0
Absence of clavicle	0	0	0	1(4.35)	0	0
Malformed offspring	0	2(8)	14(23.73)[a]	35(77.78)[a]	78(97.5)[a]	2(2.82)

Difference from the control at $p < 0.0001$.
Numerical values in parentheses are %.

Table 4. Malformations of fetuses 18 days after exposure to different doses of gamma-ray on 11.5 days of gestation.

	Dose (Gy)				
	Control	0.5	1	2	4
External malformation					
Fetus examined	69	100	110	80	60
Omphalocele	0	1(1)	0	0	0
Kinky tail	0	0	0	14(17.5)	2(3.33)
Branchyury	0	0	1(0.91)	3(3.75)	58(96.67)
Club food	0	0	0	0	12(20)
Digits	0	0	12(10.91)	72(90)	60(100)
Dwarf	0	0	0	0	60(100)
Anal atresia	0	0	0	1(1.25)	0
Hematoma	0	4(4)	6(5.45)	0	11(18.33)
Internal malformation					
Fetuses examined	35	52	58	41	32
Dilatation of cerebral ventricle	0	0	0	26(63.41)	32(100)
Stenosis of nasal cavity	0	0	0	1(2.44)	0
Cleft palate	0	0	1(1.72)	15(36.59)	27(84.38)
Levorotation of heart	0	0	0	2(4.83)	0
Abnormal lobation of lung	0	0	0	1(2.44)	0
Dilatation of renal pelvis	0	2(3.85)	0	3(7.32)	4(12.5)
Skeletal malformation					
Fetuses examined	34	48	52	39	28
Fusion of cervical	0	0	0	0	1(3.53)
Deformity of occipital bone	0	0	1(2.56)	0	4(14.29)
Splitting of cervical vertebrae	0	1(2.08)	1(1.92)	0	0
Separating of cervical vertebrae	0	2(4.17)	0	0	0
Abnormal ossification of coccygeal vertebrae	0	0	0	1(2.56)	0
Fusion of lumbar vertebrae	0	0	0	1(2.56)	0
Fusion of thoracic vertebrae	0	0	0	2(5.13)	1(3.57)
Absence of ribs	0	0	1(1.92)	0	7(25)
Fusion of ribs	0	0	0	0	6(21.43)
Wavy ribs	0	1(2.08)	4(7.69)	0	8(28.57)
Hypoplasia of ribs	0	0	0	0	27(96.43)
Displasia of sternebrae	0	0	0	1(2.56)	0
Missing of sternebrae	0	0	0	1(2.56)	0
Hypoplasia of sternebrae	0	0	0	3(7.69)	0
Curvature of tibia	0	0	0	1(2.56)	0
Absence of metatarsal bone	0	0	1(1.92)	5(12.82)	28(100)
Absence of metacarpal bone	0	0	0	15(38.46)	28(100)
Malformed offspring	0	9(9)[a]	22(20)[b]	78(97.5)[c]	60(100)[c]

[a–c] Difference from the control. [a]$p < 0.05$, [b]$p < 0.0005$, [c]$p < 0.0001$.
Numerical values in parentheses are %.

days p.c., which suggested that high-dose radiation was not proper to examine any radiation-induced abnormality of the fetal stage.

Exposure on days 5.5, 7.5 or 11.5 p.c. produced significant increases in the number of growth-retarded fetuses (GRF) (9.5, 9.2, and 13.8 folds, respectively), when compared to the control mice, though the increase was not significant after exposure during the pre-implantation (day 2.5 p.c.) periods. A significant decrease in the mean fetal weight was seen upon exposure during the organogenesis stage of days 7.5 and 11.5 p.c. (25% and 43% decrease of control mice); however, this effect was not pronounced after exposure during the fetal period, day 15.5 p.c. (Table 1). Although the embryos appeared to be sensitive to the radiation effect throughout the pre-implantation and organogenesis periods (days 5.5–11.5 p.c.), the lowest head size was recorded when exposed upon gestation, day 11.5 (Table 1). The number of growth-retarded offspring increased at 0.5 Gy, and further increased as the measured radiation dose. A similar relationship was seen in the growth parameters with a significant decrease in the mean body weight, body length and head size (Table 2). From the data summarized in Tables 3 and 4, it can be seen that a malformed fetus usually had more than one anomaly. The most common types of malformations resulting from gamma-irradiation were a cleft palate, and dilatation of the cerebral ventricle and the renal pelvis. Tail abnormalities were prominent upon exposure during the organogenesis period, especially on day 11.5 of gestation (Table 3). With increasing radiation dose, the incidence of a cleft palate, dilatation of the cerebral ventricle and abnormalities of the extremities in live fetuses rose (Table 4). Although other abnormalities were also observed in some of the exposed groups, the number of these cases was too small to indicate any causal relationship with exposure. The threshold doses of radiation that induced a cleft palate and dilatation of the cerebral ventricle, and abnormal extremities were between 1 and 2 Gy, and between 0.5 and 1 Gy, respectively.

DISCUSSION

The present work was undertaken to systematically study the comparative radiosensitivity of different gestational ages and dose-incidence relationships to acute irradiation by assessing any detectable effect in full-grown mouse fetuses. Our findings that pre-implantation exposure resulted in resorptions, while the embryos which survived the consequence developed into normal fetuses without any apparent damage agree with the conclusions derived by Russell[6,25] and Uma Devi and Baskar[20]. The maximum lethality occurred after exposure on day 2.5 p.c.; Rugh and Wohlfromm also made similar observations using X-rays[26]. Based on the stage classification of mouse development in relation to the day p.c.[27], the present results on pre-implantation exposure indicated that the morula stages (day 2.5 p.c.) had high sensitivity to a lethal radiation effect. The sensitivity to this effect decreased after day 5.5 p.c. Muller *et al*.[28] also failed to observe any significant increase in prenatal death after 1 Gy exposure on day 4 p.c. The sensitivity to radiation killing decreased as the blastocyst progressed, but again there was a period of high sensitivity during the organogenesis period, day 7.5. Irradiation at this stage resulted in a significant increase in prenatal mortality, mainly due to resorption and

embryonic death. A highly sensitive phase for mouse embryonic lethality during early organogenesis after acute exposure to 2 Gy X-rays has been reported[18]. The significant increase in the total mortality in our study after exposure on day 7.5 p.c. had a larger contribution from embryonic death than after exposure at earlier stages. The sensitivity to the lethal effects of radiation decreased during the fetal period, in agreement with reports by Konermann[18] and Rugh as well as Wohlfromm[26]. In the present study, a higher than normal prenatal mortality incidence was observed after exposure to 0.5 Gy; however, the increase became statistically significant only after 4 Gy. This supports the earlier conclusions of Russell[29] and others[7,20,30] that the period of organogenesis was less sensitive to lethal radiation effects.

Although the number of growth-retarded fetuses after gamma-exposure was high during the entire organogenesis period, the maximum retarded fetuses were produced by irradiation on day 11.5 p.c. In this study, we observed the abnormalities in the fetuses and some data presented that the results were similar when litter effects were considered[31]. A significant reduction in the mean fetal weight was also seen in fetuses exposed during the later period of organogenesis, days 7.5–11.5 p.c. This result is in agreement with the findings of Konermann[18], Russell[8] and Kriegel *et al*.[32], that the largest loss of weight was brought about by irradiation on day 10 or 11 p.c. Exposure during the fetal stage of day 15.5 p.c. also resulted in a significantly lower fetal weight, indicating that susceptible fetuses at this stage were as vulnerable to the stunting effect of radiation as at the later organogenesis period, but a comparatively smaller number was affected. It is of interest to note that a small head size was a prominent effect in Japanese children exposed to irradiation during 4–17 weeks of gestation[33], and that a significant decrease in head size was a feature observed after irradiation of mice at day 11.5 p.c. with both x-rays and gamma-rays[19,34]. In the present study, a noticeable decrease in head size (both length and width) was also evident after exposure at days 5.5–15.5 p.c., but the maximum shortening of head was seen after exposure on day 11.5 p.c. The head width was also similarly reduced after exposure at this stage. An increase in the number of growth-retarded offspring was seen at 0.5 Gy, which increased further as the radiation dose increased. A similar effect was seen in the growth parameters, including a significant decrease in the mean body weight, body length and head size.

The most common prominent types of malformations resulting from gamma-irradiation during the organogenesis period, especially on day 11.5 of gestation, were a cleft palate, dilation of the cerebral ventricle and renal pelvis, and abnormalities of the extremities and tail. The abnormalities of extremities were brachydactyly, ectrodactyly, polydactyly, and syndactyly, which would be severe defects in postnatal mice[35]. In addition, a cleft palate, dilatation of the cerebral ventricle and abnormalities of the extremities in live fetuses rose with increasing radiation dose. The data presented in Tables 3 and 4 indicate that a malformed fetus usually had more than one anomaly. Some mice, especially those irradiated with high doses, had many abnormalities on the same forepaw(s) and/or hindpaw(s). Here, the fetuses which had many abnormalities on the foreleg and /or hindleg were counted as one. The number of fetuses with abnormal extremities was significantly higher in those groups exposed to radiation at a dose of 1 Gy or more. The abnormalities of the extremities after irradiation were more frequent than a cleft palate. These results are in agreement with earlier studies[35–37] that the maxi-

mal frequency was found after exposure during the organogenesis period, and a dose-dependant increase was observed during this period. Although other anomalies were observed in some of the exposed groups, the number of these cases was too small to consider to have a causal relationship with exposure.

In conclusion, the results presented in this study indicated that the late organogenesis period was a particularly sensitive phase in the development of the brain, skull and extremities of mouse. The threshold doses of radiation that induced a cleft palate and dilatation of the cerebral ventricle, and abnormal extremities were between 1 and 2 Gy, and between 0.5 and 1 Gy, respectively. In the present study, we excluded any strain variation because further study is underway concerning the genetic difference that is responsible for a malformation.

ACKNOWLEDGEMENT

This work was supported by the Nuclear R&D Program from the Ministry of Science and Technology of Korea.

REFERENCES

1. Brent, R. D. (1984) The effects of ionizing radiation, microwaves, and ultrasound on the developing embryo: Clinical interpretations and applications of the data. Curr. Proble. Pediatr. **14**: 1–87.
2. Committee on the biological effects of radiation (1980) The effects on populations of exposure to low levels of ionizing radiation, 1980, p. 480, National Academy Press, Washington.
3. Beir, V. (1990) Health effects of exposure to low levels of ionizing radiation. *In*: Biological effects of ionizing radiations. pp. 22–45, National Academy Press, Washington
4. Aolad, H. M., Inoue, M., Darmanto, W., Hayasaka, S., and Murata, Y. (2000) Hydrocephalus in mice following X-irradiation at early gestational stage: possible due to persistent deceleration of cell proliferation. J. Radiat. Res. **41**: 213–226.
5. Roy, K., Kodama, S., Suzuki, K. and Watanabe, M. (1999) Delayed cell death, giant cell formation and chromosome instability induced by X-irradiation in human embryo cells. J. Radiat. Res. **40**: 311–322.
6. Molls, M., Zamboglou, N. and Streffer, C. (1983) A comparison of the cell kinetics of pre-implantation mouse embryos from two different mouse strains. Cell Tissue Kinet. **16**: 277–283.
7. Muller, W. U. and Streffer, C. (1986) Changes in frequency of radiation induced micronuclei during interphase of four cell mouse embryos in vitro. Radiat. Environ. Biophys. **25**: 195–199.
8. Russell, L. B. (1950) X-ray induced developmental abnormalities in the mouse and their use in the analysis of embryological patterns. I. External and gross visceral changes. J. Exp. Zool. **114**: 545–601.
9. Rugh, R. and Grupp, E. (1959) Exencephalia following X-irradiation of the preimplantation mammalian embryo. J. Neuropathol. Exp. Neurol. **18**: 468–481.
10. Konermann, G. (1969) Die Keimesentwicklung der Maus nack Einwirkung kontinuierlicher Co^{60} Gamma-bestrahlung wahrend der Blastogenese, der Organogenese und Fetal periode. Strahlentherapie **137**, 451–466.
11. Hande, M. P., Uma Devi, P. and Jagetia, G. C. (1990) Effect of in utero exposure to low doses of low energy X-rays on the postnatal development of mouse. J. Radiat. Res. **31**: 354–360.
12. Hicks, S. P. (1953) Developmental malformations produced by radiation. Am. J. Roentgenol, **69**: 272–293.
13. Wilson, J. G., Jorden, H. C. and Brent, R. L. (1953) Effects of irradiation on embryonic development. II. X-rays on the 9th day of gestation in the rat. Am. J. Anat. **92**: 153–188.

14. Murakami, U., Kameyama, Y. and Nogami, H. (1963) Malformation of the extremity in the mouse foetus caused by X-radiation of the mother during pregnancy. J. Embryol. Exp. Morphol. **11**: 540–569.
15. Ohzu, E. (1965) Effects of low dose X-irradiation on early mouse embryos. Radiat. Res. **26**: 107–113.
16. Jacobsen, L. (1965) Low-dose embryonic X-radiation in mice and some seasonal effects in the perinatal period. Radiat. Res. **25***:* 611–625.
17. Michel, C. and Fritz-Niggli, H. (1978) Late biological effects of ionizing radiation. Vienna, IAEA, vol. 2, pp. 397–408.
18. Konermann, G. (1987) Post implantation defects in development following ionizing irradiation. Adv. Radiat. Biol. **13**: 91–167.
19. Uma Devi, P. Baskar, R. and Hande, M.P. (1994) Effect of exposure to low-dose gamma radiation during late organogenesis in the mouse foetus. Radiat. Res. **138**: 133–138.
20. Uma Devi, P. and Baskar, R. (1996) Influence of gestational age at exposure on the prenatal effects of γ-radiation. Int. J. Radiat. Biol. **70**: 45–52.
21. Mole, R. H. (1992) Expectation of malformations after irradiation of the developing human in utero: The experimental basis for predictions. Adv. Radiat. Res. **15**: 217–301.
22. United Nations Scientific Committee on the Effects of Atomic Radiation (1986) Genetic and somatic effects of ionizing radiation. pp. 263–366, United Nations, New York.
23. Wilson, J. G. (1965) Embryological considerations in teratology. *In*: Teratology: principles and techniques (Wilson JG and Warkaney J, ed.), pp. 251–261, The University of Chicago Press, Chicago.
24. Kimmel, C. A. and Trammell, C. (1982) A rapid procedure for routine double staining of cartilage and bone in fetal and adult animals. Stain. Technol. 56: 271–273.
25. Russell, L. B. (1981) Utilization of critical periods during development to study the effects of low levels of environmental agents. *In*: Measurement of risks (Berg GG and Maillie HD, ed.), pp. 143–162, Plenum, New York.
26. Rugh, R. and Wohlfromm, W. (1962) Can the mammalian embryo be killed by X-irradiation? J. Exp. Zool. **151:** 227–244.
27. Nakatsuji, N. (1992) Development of postimplantation mouse embryos: Unexplored field rich in unanswered questions. Dev. Growth Differentiation **34**: 489–499.
28. Muller, W. U., Streffer, C. and Pampfer, S. (1994) The question of threshold doses for radiation damage: malformations induced by radiation exposure of unicellular or multicellular preimplantation stages of mouse. Radiat. Environ. Biophys. **33:** 63–68.
29. Russell, L. B. (1954) The effects of radiation on mammalian prenatal development. *In*: Radiation biology, (Hollaender A, ed.), pp. 861–918, McGraw-Hill, New York.
30. UNSCEAR (1977) Sources and effects of ionizing radiation. pp.655–725 United Nation, New York.
31. Devi, P. U., Baskar, R. and Hande, M. P. (1994) Effect of exposure to low-dose gamma radiation during late organogenesis in the mouse fetus. Radiat. Res. **138**: 133–138.
32. Kriegel, H., Langendorff, H. and Shibata, K. (1962) Die Beeniflussung der Embryonalentwiklung bei der Maus nach einer Rontgenbestrahlung. Strahlentherapie **119**: 349–370.
33. Miller, R. W. and Blot, E. J. (1972) Small head size after *in utero* exposure to atomic radiation. Lancet **2:** 784–787.
34. Uma Devi, P. and Hande, M. P. (1990) Effect of low dose 70 kVp X-rays on the intrauterine development of mice. Experimentia **46**: 511–513.
35. Kusama, T., Sugiura, N., Kai, M. and Yoshizawa, Y. (1989) Combined effects of radiation and caffeine on embryonic development in mice. Radiat. Res. **117**: 273–281.
36. Cekan, E., Tribukait, B. and Vokal-Borek, H. (1985) Protective effect of selenium against ionizing radiation-induced malformations in mice. Acta. Radiol. Oncol. **24**: 267–271.
37. Cekan, E., Slanina, O., Bergman, K. and Tribukait, B. (1985) Effects of dietary supplementation with selenomethionine on the teratogenic effect of ionizing radiation in mice. Acta. Radiol. Oncol. **24:** 459–463.